A39

Preparation of solid DNA nanoparticles for use in gene therapy

Martina Hanzlíková[1,*], Janne Raula[3], Juho Hautala[3], Esko Kauppinen[3], Arto Urtti[2], Marjo Yliperttula[1]

[1] *Division of Biopharmaceutics and Pharmacokinetics, Finland*

[2] *Centre for Drug Research, Faculty of Pharmacy, University of Helsinki, Viikinkaari 5E, FI-00014 Helsinki, Finland*

[3] *NanoMaterials Group, Department of Applied Physics, Aalto University, P.O. Box 15100 FI-00076 Aalto, Finland*

*Corresponding author.

E-mail: martina.hanzlikova@helsinki.fi (M. Hanzlíková).

Background: Non-viral gene therapy, based on nanosized particles, is a potential therapeutic option in various diseases. The success is mainly dependent on an efficient gene delivery vector. Aerosol synthesis can provide pure solid DNA particles with substantial high dose of DNA per particle and thereby increase the amount of DNA delivered into cells as compared to commonly used DNA polymer complexes. The purpose of this study was to test the suitability of plasmid DNA alone or in complex with cationic polymers for the preparation of solid DNA nanoparticles by an aerosol flow reactor method. *Methods*: The sample solutions contained either plasmid DNA (pDNA) alone or complexed at a ratio of 1:1 (w/w) with branched or linear polyethylenimine (PEI) with the molecular weight of 25 kDa. The additive agents, L-leucine and mannitol, were added to PEI/DNA complexes at a ratio of 1:8 (w/w). The aerosol flow reactor method [1] involved atomization of sample solutions to nanosized droplets, which were immediately dried in a heated flow reactor tube by the evaporation of the solvent. The dried nanoparticles were collected with a low-pressure impactor and the size distribution was determined by a differential mobility analyzer. The surface morphology was analyzed using field emission scanning electron microscopy and the structural integrity of pDNA was evaluated by agarose gel electrophoresis. *Results*: The produced pure pDNA nanoparticles were spherical and had a mean diameter of 125 nm. However, pDNA in such nanoparticles did not preserve its supercoiled structure due to the shearing stresses caused by the atomization process. The complexation of pDNA with PEIs before atomization allowed the maintanence of pDNA integrity. The further addition of either L-leucine or mannitol to initial sample solution, stabilized nanoparticles structure and prevented them from water uptake and subsequent deformation. The resulting solid nanoparticles had a mean size between 65 and 125 nm and the loading content of pDNA in a single nanoparticle was approximately 10% (w/w). *Conclusions*: The aerosol flow reactor method provides an effective way of producing solid DNA nanoparticles with a size optimal for cell uptake and for potential use in non-viral gene delivery

Reference

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A40

Exploiting a bacterial toxin translocation domain for the endosomal escape of CPP-imported cargoes

Arshiya F. Mohammed*, Amaalia E. Broad, Jean Gariepy*

Department of Medical Biophysics, University of Toronto, Sunnybrook Research Institute, 2075 Bayview Avenue, M4N3M5 Toronto, Ontario, Canada

*Corresponding author.

E-mails: arshiya.mohammed@utoronto.ca (A.F. Mohammed), gariepy@uhnres.utoronto.ca (J. Gariepy).

The clinical impact of CPP-based delivery agents has yet to be realized due to a lack of delivery efficiency to the cell cytoplasm. Polycationic cell penetrating peptides are a major class of CPPs. However, upon internalization via endocytosis, these CPPs are typically trapped in endosomes and are subsequently degraded or recycled out of cells. To promote endosomal escape, we investigated the use of a bacterial protein domain derived from *Pseudomonas aeruginosa*, Exotoxin A (ETA253-412), capable of translocating known protein domains out of vesicular compartments. We constructed, expressed, and purified a series of CPP–ETA253-412–eGFP fusion proteins. We used confocal microscopy and flow cytometry to confirm the internalization of CPP (poly-arginine or TAT)-containing constructs at 37°C in human cervical carcinoma (HeLa) cells. Additionally, we observed the time-dependent relocation of CPP-ETA253-412–eGFP constructs from the endosome to the cytosol. These experiments demonstrate the potential of the ETA253-412 translocation domain in relocating cargoes, such as protein therapeutics, siRNA and vaccine formulations, to the cytosol of target cells.

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A41

Investigation of microsphere-mediated cellular delivery

R.M. Sanchez-Martın*, L.M. Alexander, S. Pernagallo, A. Livigni, J.M. Brickman, M. Bradley

Chemical Biology Section, School of Chemistry & Institute of Stem Cell Research, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, UK

*Corresponding author.

E-mail: rosario.sanchez@ed.ac.uk (R.M. Sanchez-Martın).

Recently we have developed a polystyrene microsphere-based system designed to efficiently deliver biological materials into a broad range of cell lines [1,2]. This versatile delivery system is capable of transporting any biological cargo from small molecules to oligonucleotides and bulky proteins into cells [3–5]. However, the specific mechanism of cellular entry is largely unknown and widely varies from study to study. As such, chemical, biological and microscopic methods have been used to elucidate the mechanism of cellular uptake for these nanoparticles in several cell lines. Additionally, gene expression profiling has been used to determine if there is a transcriptional response to 'beadfection' [6,7].

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